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A Systematic Approach Review on Method Development and Validation of Tyrokinase Inhibitors by RP-HPLC

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ABSTRACT

Cancer incidence would more than double, according to the new World Cancer Report. New, more successful cancer treatments are enabled through advances in tumor biology and molecular genetics. Drug targeting signaling was designed to target the pathways' hubs. FDA has licensed 43 RTK inhibitors (RISUG inhibitors) for oncological indications starting in August 2019. Many reversible inhibitors do not bind to or close the adenosine triphosphate binding site, making them removable (ATP) ATP-competitive inhibitors are the bulk (type-I inhibitors) LC is an extremely effective analytical instrument in chemistry. The HPLC is the most reliable tool that is often used for both quantitative and qualitative studies of pharmaceutical products. Sample preparation is a crucial step in the production process. Processed samples are created to increase the accuracy of sample analysis. Almost all optimization of HPLC method production has relied on optimizing HPLC conditions. Validation is necessary when an experimental or changed procedure is performed in two or more laboratories by separate operators, all using the same equipment. Recommended values FDA, USP, and ICH have are: Reproductivity is one type of precision. The accuracy of measurement is generally indicated as a normal deviation or relative standard deviation.

INTRODUCTION

According to the latest World Cancer Report, the global cancer rate will increase to 18 million by 2025, with 21.4 million new cancer cases and 13.2 million deaths expected by 2030[1-2]. Modern, more effective drug therapies are being created as a result of advancements in tumor biology and molecular genetics [3, 4]. All those involved in cancer research and patient care face many obstacles. Cancerous cells divide uncontrollably and infiltrate the blood and lymphatic systems, spreading to other areas of the body. A single tumor cell that is surrounded by normal cells replicates more rapidly. When a small cancerous mass develops, normal cells are unable to compete with tumor cells for nutrients in the bloodstream. Before the tumor reaches its maximum diffusion rate, tumor cells will begin to transfer normal cells [4, 5]. Although the external surface of the mass readily absorbs nutrients, the internal cells form a necrotic center within the tumor, depending on diffusion to acquire nutrients and eliminate waste products. Thus, a stable state tumor size grows before a more connected circulatory system. It will last until the tumor regains circulation [5], which could take years. There are over a hundred distinct types of cancer. Cancers include bladder cancer, breast cancer, colorectal cancer, lung cancer, and prostate cancer, among others. The majority of human malignancies are characterized by changes in normal cell physiology, but six critical changes are as follows: [6, 7]

1. Self-sufficiency in growth signals

2. Anti-growth sensitivity

3. Invasion and Metastasis of Tissue

4. Unlimited replication potential

5. Persistent angiogenesis

6. Resisting apoptosis

TYROKINASE INHIBITORS

The proliferation of cancer cells in Darwinian has been proposed to maintain tumor microenvironment (TME) conditions and Darwinian selection improvements [8]. Intracellular drug targeting signaling was developed to target the molecular pathways' hubs. Among these drugs, inhibitors of receptor tyrosine kinase (RTK) comprise a large family of these targeted

therapies that have been clinically used with varying degrees of success since 2001. On the active site of the kinase, phosphorylation of intracellular targets often involved in cell proliferation or angiogenesis is generally prevented [9,10]. Since August 2019, the Food and Drug Administration (FDA) has approved 43 RTK inhibitors for oncological indications [11]. In general, reversible inhibitors are distinguished from irreversible inhibitors by their ability to covalently attach to or close the adenosine triphosphate binding site (ATP). The majority of non-covalent inhibitors are ATP competitive inhibitors (type-I inhibitors). Since ATP binding sites are frequently conserved, selectivity can be achieved by focusing on poorly conserved residues, especially hinge residues. Type-II inhibitors bind to and stabilize the inactive conformation of inactive kinases near their ATP binding site. This form of inhibitor is usually non-selective. Allosteric inhibitors (type III) inhibit kinase with high selectivity by binding to an Allosteric site distinct from the ATP and hinge sites [12]. New substratum-directed inhibitors or Type IV RTKIs that target a reversible substratum site are being created. Finally, covalent Kinase inhibitors, also known as type V inhibitors, are irreversibly linked to the kinase activity site and exhibit significant off-target effects. [13]

INSTRUMENTATION

Liquid chromatography is one of the most powerful analytical techniques available in chemistry today. It is capable of isolating, categorizing, and quantifying dissolved substances in any sample stream. The most precise analytical technique is high-performance liquid chromatography, which is frequently used for both quantitative and qualitative assessments of pharmaceutical goods. [14] A sample solution is injected into a porous column (stationary phase) and a liquid (mobile phase) is pumped through the column at high pressures, according to the theory. The sample division was made based on the changes in migration rates between the stationary and mobile phases generated by the various sample divisions. Elution happens at varying rates depending on the activity of component partitioning. [15] The sample compound that has a higher affinity for the stationary layer travels more slowly than the sample compound that has a lower affinity, which travels faster and further. [16] The advantages of high-performance liquid chromatography are enhanced by the fact that it is not limited to fluid or thermally stable materials and that a wider selection of mobile and stationary phases are accessible. [17]

METHOD DEVELOPMENT [18, 19]

- Sample preparation
- Method optimization
- Method validation

Sample preparation: The analyst must complete the sample preparation process as part of the production process. For each analysis technique used for a specific in-process sample or dosage type for subsequent HPLC analysis, the sample preparation method should be properly defined. The manufacturer, filter type, and pore size of the filter media must be calculated for the analytical process. [20] Sample preparation aims to establish a processed sample that, when combined with the original sample, yields more accurate analysis results. Aliquots should be prepared using as few HPLC-compatible interfaces as possible and without causing column damage. [21–23]

Method optimization: The majority of optimizations in the development of HPLC methods have focused on optimizing HPLC conditions. In the liquid chromatography (LC) optimization procedure, the primary control variables are the various components of acidity, solvent, gradient, fluctuating temperature, sample volumes, and diluents solvent type determination in the mobile phase. This is used to evaluate the optimal combination of resolve and analytical time following efficient selection. We considered column size, particle size, and column packing based on flow rate. These parameters are adjustable regardless of ability level or range.

Method Validation: Every new or changed technique must be validated to ensure reproducible and consistent outcomes when conducted in the same or different laboratories by different operators using the same equipment. The validation method that is needed is entirely dependent on the process that is being validated and the applications that are being proposed. Method validation results may be used to ascertain the precision, reliability, and consistency of study findings; these are essential components of any successful analysis. The method validation process necessitates the use of properly balanced and specification-based equipment. Methods of analysis must be tested or revalidated. [25–27]

Specificity: Selectivity in analytical methods is described as the degree to which an analytical method can measure the analyte, when interferences are present, with absolute accuracy. [28]

Linearity and range: The ability of an analytical method to obtain test results that are directly proportional to the concentration of the sample analyte is referred to as linearity (within a defined range). A linear relationship can be evaluated across the entire empirical spectrum. Typically, linearity is expressed as the slope of the regression line. [26–28] For linearity, the ICH recommends a minimum of five concentrations. [29]

Precision: The degree of agreement (degree of scattering) between a series of measurements made under defined conditions with multiple samples from the same homogeneous specimen is referred to as the precision of a process. Repeatability, moderate precision, and reproductivity are three distinct levels of precision [29]. Usually, research precision is expressed in terms of the standard deviation or relative standard deviation of the measurement sequence. Precision may refer to an analytical process's reproducibility or recurrence under normal operating conditions. The word "medium accuracy" (i.e. "roughness") refers to differences between laboratories on different days or between analysts or equipment within a single laboratory.

Accuracy (Recovery): The degree of correspondence between a value known as a standard true value or an agreed-upon reference value and the value discovered indicates the analytical method's accuracy. It is calculated using the same sampling technique as the analyte concentrations. These can be examined using normal and blank solutions to ensure that there is no need for intervention. The accuracy is then expressed as a percentage of analytes fully recovered from the test results. Additionally, it can be expressed as a recovery by conducting tests on additional analyte concentrations that are already present. [28, 29]

Solution stability: When conducting validation and storage tests under normal conditions and storage conditions, the standards and samples' stability are determined as well as when, in certain cases, they are measured on the instrument to determine whether additional measures, such as climate control or light safety, are needed.

Limit of detection (LOD): However, a very limited quantity of measurement (not an exact number) is done on a sample. The signal-to-noise (S/N) ratio used in an analytical technique, such as an analysis of the concentration of an analyte in a sample, can be between 3:1 (it is

calculated using the amount of the analyte present in the sample). A maximum height of a component, or part's maximum height, is called "H." This is also known as the "signal-to-noise ratio." h = the absolute value of the largest difference between the chromatogram's baseline and the sound used to collect data. [28-30]

Limit of Quantification (LOQ): A quantitation limit is an analysis method that is defined as the smallest amount of analysis in a sample that can be quantified accurately and precisely. In analytic procedures, like HPLC, with base noise, the LOQ is usually by calculating the S/N ratio (10:1) and is then checked by injection criteria and provides an appropriate relative percentage defect. [29, 30]

Robustness: A system's capability to keep its steady, stable characteristics despite small but deliberate parameter alterations (e.g. pH, mobile phase composition, temperature, and instrumental adjustments). [28, 29]

System Suitability: The device was calibrated before beginning the study to ensure that its detection sensitivity, resolution, and reproducibility were optimized. Since it is assumed that all of the instruments, electronics, analytical processes, and samples to be tested are all integrated into a single device, which can be measured, it follows that every instrument, electronics, analytical process, and sample has been integrated into the test device. Applying the approach involves determining a variety of test parameters, including peak resolution, theoretical plate numbers, peak tailing, and applicability. [26-30]

Table No. 1: OVERALL REVIEW ON TYROKINASE INHIBITORS USING RP-HPLC

S. NO	TITLE	COLUMN	MOBILE PHASE	FLOW RATE	DETECTION WAVELENGTH	INJECTION VOLUME	TARGET GENE	REFERENCE
1	**A New Simple Method Development and Validation of**	Develosil ODS HG-5 RP C18, 5µm, 15cmx4.	0.1% Orthophosphoric Acid:	1.0mL/min	287nm	20µl	Bruton's tyrosine kinase	31

	Ibrutinib In Bulk and Pharmaceutical Dosage Form By RP-HPLC	6mm id column	Methanol with 35: 65 ratio				receptor (BTK)	
2	**Separation and Estimation of process-related Impurities of Gefitinib by Reverse-Phase High-Performance Liquid Chromatography**	Inertsil ODS-3V column (250 × 4.6 mm i.d.; particle size 5 µm)	Mobile phase 130 mM ammonium acetate and acetonitrile (50:50, v/v)	1.0 ml/min	260nm	20µl	Epidermal growth factor receptor (EGFR)	32
3	**RP-HPLC method development and validation for estimation of Alectinib in bulk and pharmaceutical dosage form**	zodiacal 150mm x 4.6mm, 5µm	Water; Acetonitrile (50:50v/v)	1.0ml/min	265nm	10µl	ALK (Anaplastic lymphoma kinase)	33
4	**Development and Validation of RP-HPLC Method of Cabozantinib in Active Pharmaceutical Ingredient and Pharmaceutical Dosage form**	C18 column 4.6 x 250 mm, 5µm.	Methanol: phosphate buffer (ph. 3.00) with orthophosphoric acid (OPA) (55:45 % v/v)	0.8ml/min	244nm	20µl	Inhibitor of the tyrosine kinases c-Met andVEGFR2	34
5	**RP-HPLC Method Development And Validation For The Determination Of Lorlatinib In Bulk**	Eclipse plus C18 (250mm X 4.6mm, 3µm)	Potassium dihydrogen orthophosphate, acetonitrile,	1.0ml/min	310nm	10µl	Anaplastic lymphoma kinase (ALK)	35

	And Its Pharmaceutical Formulation		and methanol (50:30:20V/V)					
6	**Stability indicating RP-HPLC method development and validation for the determination of Acalabrutinib in bulk drug and capsule dosage form**	Zodiasil C18 150 mm x 4.6 mm, 5 □m column	(Water and methanol 60:40%v/v)	0.8ml/min	230nm	10µl	Bruton Tyrosine Kinase(BTK)	36
7	**A New Stability Indicating High-Performance Liquid Chromatography Method for the Estimation of Ruxolitinib in Bulk and Tablet Dosage Form**	ODS RP C18, 250mm x 4.6mm .i.d., 5µm column	A mixture of acetonitrile: methanol: 1% orthophosphoric acid (70:25:5)	1.0ml/min	258nm	20µl	Janus kinase (JAK) inhibitor	37
8	**Development and Validation of RP-HPLC Method for the Estimation of Nilotinib in Bulk and Pharmaceutical Dosage form**	Phenomenex enable C18 column (15x4.6mm, 5µm particle size)	Acetonitrile and phosphate buffer pH 5) at the proportion of 60:40 %v/v	1.0ml/min	260nm	20µl	selective BCR-ABL inhibitor	38
9	**Development And Validation of RP-HPLC Method For The Determination**	C18 column (4.6 mm i.d. × 250 mm, 5 µm particle size)	Methanol and acetonitrile mixed in	1.0ml/min	323nm	20µl	BCR/Abl (the "Philadelphia	39

	of Dasatinib In Tablet Dosage Form		the ratio of 50:50 v/v,				chromosome"), Src, c-Kit, ephrin receptors	
10	**Development and Validation of an RP-HPLC Method for Vemurafenib in Human Urine**	X-Terra RP-18 column (250 x 4.60 mm, ID 5 µm)	Acetonitrile : water 60:40 (v/v)	1.0ml/min	249nm	10µl	BRAF(V600E) kinase inhibitor	40
11	**Exploring RP-HPLC Method for analysis of Axitinib in Bulk and in-house Tablets**	BDS C18 (250 mm × 4.6 mm, 5 µm).	methanol: water 85:15% v/v	1.0ml/min	330nm	20µl	vascular endothelial development factor receptors (VEGFR-1, VEGFR-2, VEGFR-3)	41
12	**Method Development And Validation of RP-HPLC For Assay of Trametinib In Pharmaceutical Dosage Form**	Zodiac C18, 250×4.6mm ID, 5µm Particle size	Phosphate Buffer: Methanol (40:60)	1.0ml/min	257nm	20µl	MEK1 and MEK2	42
13	**Analytical Method Development and Validation of Vandetanib by Using**	C18 column (Inerstil ODS-3V -5 um 250×4.6 mm)	methanol (100 v/v)	1.0ml/min	328nm	10µl	Epidermal growth factor receptor	43

	RP-HPLC of Bulk Drug						(EGFR) and RET inhibition, EGFR-2 (VEGFR-2)	
14	**Development And Validation Of An RP-HPLC Method For Bosutinib In Bulk Form**	C-18 column (250mm x 4.6mm i.d., 5µm particle size)	methanol and Sodium Phosphate Buffer 10 mm ph 6.5 mixed in a proportion of 85:15 v/v	0.7ml/min	266nm	20µl	SRC and ABL kinases,	44
15	**Development and Validation of a New Chromatographic Method for the Estimation of Vismodegib by RP-HPLC**	column C18, (150 × 4.6) mm, 5 µm	0.1% phosphoric acid and Acetonitrile in the proportion of 50:50 (v/v)	1.0ml/min	264nm	10µl	Hedgehog signal transduction	45
16	**Development and Validation of a Stability Indicating RP-HPLC Method for the Determination of Nilotinib (A Tyrosine Kinase Inhibitor)**	C18 (250mm × 4.6mm i.d., 5 µm particle size) column	Water: acetonitrile: glacial acetic acid (20: 80: 0.03, v/v).	1.0ml/min	254nm	20µl	BCR-ABL protein	46
17	**A Validated Stability Indicating RP-HPLC Method for the**	Phenomenex Luna-C18 column (4.5x250	Methanol: acetonitrile: water	1.0ml/min	275nm	10µl	VEGFR2 -TIE2	47

	Estimation of an Anti-Cancer Drug Regorafenib In Pure and Pharmaceutical Dosage Form	mm; 5 µm particle size)	(55:25:20 v/v/v)					

CONCLUSION

There have been various methods applied for the qualitative evaluation of anticancer drugs. This review will include a comprehensive review of the literature on the process production and validation of tyrokinase inhibitors. This will provide a foundation for researchers working in the areas of product creation and product testing.

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